



Aceh International Journal of Science and Technology

ISSN: 2088-9860

Journal homepage: <http://jurnal.unsyiah.ac.id/aijst>



A General Overview on Some Aspects of Fish Reproduction

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Received : March 1, 2014

Accepted : April 29, 2014

Abstract - Reproduction is one of the important physiological systems that are crucial in the life cycle of living organisms including fish. The main objective of the reproduction is to maintain the existence of the species and therefore fish have a strategies and tactics to achieve this objective. The reproduction behaviours are important to be studied in relation to know the population dynamic of fishes and their spawning seasons. This information is very crucial in relation to the development of breeding technology for aquaculture and conservation (restocking) purposes. This paper reviews the reproductive strategy, fecundity and spawning frequency of fishes.

Keywords: Fecundity; Spawning frequency; Gonadal development; Synchronous; gonadosomatic index

Introduction

In general the reproduction can be defined as a biological process of living organism to inherit the properties of its parent to their offspring in order to ensure the continuing survival of the concerned species. In fish, there are some tactics and strategies used by fish to ensure their offspring survive.

Studies on reproductive biology of fish are crucial needed and a basic requirement to plan a better conservation and management strategies of fishery resources (Ali and Kadir, 1996; Ezenwaji *et al.*, 1998; Brewer *et al.*, 2008; Grandcourt *et al.*, 2009; Muchlisin *et al.*, 2010), examination of basic life-history information and for evaluating the impacts of environmental variability on the dynamics of fish populations (Schlosser, 1990). In addition, information on the reproductive system is essential for the development of the commercial aquaculture of an aquatic species (Muchlisin, 2004). Natural challenge leads the fish to the maximization of the lifetime production of offspring, and more importantly to maximization of survivorship of offspring until adulthood (Murua and Sabodiro-Rey, 2003). Studies on the reproduction biology are a popular topic in last decades, beside for conservation purposes, information on reproduction biology is also useful to select the candidate of fish target from the wild for diversification of fish species in aquaculture industry (Muchlisin, 2013). Herein, we reviewed and summarized some aspects of reproductive biology of fishes such as reproductive strategy, fecundity and spawning frequency.

Reproductive Strategy

The reproductive strategies of fishes are often reflected in the anatomical differences between the sexes; male and female. The objective of a reproductive strategy is to maximize reproductively active offspring in relation to available energy and parental life expectancy (Roff, 1992), fish take different strategies and tactics to achieve this objective (Balon, 1984). The reproductive strategy of a fish species is the overall pattern of reproduction common to individuals of within species, whereas the reproductive tactics are those variations in response to

fluctuations in the environment (Wootton, 1990; Roff, 1992). Knowledge on the reproductive behavior of fishes is necessary for the development of the commercial aquaculture industry of an aquatic species in general.

The reproduction organ of fish can be classified as testis for males and ovary for females. The sexes of most fishes can be distinguished by examination of the gonad. Both the testes and ovary are typically paired structures that are suspended by mesenteries across the roof of the body cavity, in close association with the kidney (Moyle and Cech, 2000).

Teleost fishes have a diversity of sexual patterns and gonadal morphology, particularly in the appearance of female and male tissue within gonads (Asoh and Kasuya, 2002) and some species have sexual dimorphism between male and female. Redding and Patino (1993) cited that fish gonad consists of germ cells, which produce gametes and somatic cells, which functions to support, nourish, and regulate the development of germ cells. Gonadal ducts are present in most species to carry gametes to their appropriate internal or external destinations. The majorities of teleosts are oviparous and much more fecund than elasmobranchs. The oviparous fishes are mostly external fertilization, while the fish with viviparous mode usually had internal fertilization, for example in killifishes, Poeciliidae (Bone *et al.*, 1996), elasmobranchs and *Sebastes* sp. (Murua and Saborido-Rey, 2003). The oviparous fishes can be classified as broadcast spawning, demersal non-guarding, demersal guarding and brooders.

Some species of fish had a hermaphroditism mode, but most of the fish are gonochoristic. In general the hermaphroditism can be divided into three categories; synchronous, protandrous and protogynous. The synchronous hermaphrodite is a species which can simultaneously produce eggs and sperm, and protandrous hermaphrodite is a fish starts as a male and later may switch to female, while the protogynous hermaphrodite is a fish begins reproductive life as a female and later may switch to male (Avisé and Mank, 2009). The synchronous hermaphroditism was detected in *Serranus cabrilla*, and the protandrous was recorded in *Sparatus auratus*, *Sarpa salpa*, *Acanthopagrus berda* and *Lithognathus mormyrus* while some species of groupers (*Epinephelus* spp.) displayed a protogynous hermaphroditism.

The ovaries of adult fish exist as paired structures attached to the body cavity on either side of the dorsal mesentery. Three patterns of ovarian development are generally observed in fishes, i.e. synchronous, group synchronous, and asynchronous.

- (a) Synchronous pattern; in the synchronous category, all of oocytes develop and ovulate in unison and there is no replenishment from early stage, and a single size distribution in the ovary (West, 1990), for example in common carp (*Cyprinus carpio*).
- (b) Group synchronous pattern; this group has at least two populations of oocytes at any developmental stages (Murua and Saborido-Ray, 2003). This pattern allows for multiple, distinct ovulatory events that typically follow seasonal, lunar, or diurnal cycles (Redding and Patino, 1993). This pattern was documented in some fishes for example rainbow selebensis, *Telmatherina celebensis* (Nasution, 2005), white mullet *Mugil curema* (Solomon and Ramnarine, 2007), and tucunare *Cichla kelberi* (Normando *et al.*, 2009).
- (c) Asynchronous pattern; ovaries showing the asynchronous pattern contains oocytes at all stages of maturity without dominant populations. The ovary appears to be a random mixture of oocytes at every conceivable stage, allowing for protracted or continuous ovulation (Redding and Patino, 1993; Murua and Saborido-Rey, 2003), for instance pouting *Trichopterus luscus* (Alonso-Fernandez *et al.*, 2008) and *Cichla kelberi* (Normando *et al.*, 2009).

Fish with synchronous pattern are known as total spawner or , where the whole clutch of yolked oocytes ovulates at once and the eggs are shed in over a short period of time. While, a fish with asynchronous ovulator is known as batch spawner or multiple spawner, where only a portion of the yolked oocytes is spawned in each batch, usually through the hydration process (Murua and Saborido-Rey, 2003). Batch spawning is a strategy to release eggs over a long period of time increasing the survival probability of offspring (Lambert and Ware, 1984). In addition, a group synchronous is known as a fractional multiple spawners, distinct ovulatory events that typically

follow seasonal, lunar, or diurnal cycles (Redding and Patino, 1993). Some species showing the semelparity pattern that fish spawned once time during their life cycle before death, for example Pacific salmon (*Oncorhynchus* spp.), however most of fish have an iteroparity reproductive pattern.

In generally fish released the eggs into waters. In order to maximize reproductive success, some fish have produced eggs with attachment apparatus called as villiform, especially was occurred in freshwater fish, while some species such as gouramis and grass carp produce buoyant eggs, not floating kinds maybe best suited to freshwater, to proven excessive drift in rivers and streams. While marine fish eggs are usually smaller in diameter than freshwater fish and most always buoyant and liberated into the pelagic zone (Bone *et al.*, 1996). The villiform is an adhesive filament and purposes of this organ is to attach to the substrates when the eggs are released into water and protect them from being washed away in the water. Fish with an attachment apparatus organ include in *Lepadogaster lepadogaster*, *Diplecogaster bimaculatus* and *Apletodon dentatus* (Breining and Britz, 2000), *Polypterus ornatipinnis*, *Erpetoichthys calabaricus*, *Polypterus senegalus*, *P. ornatipinnis* (Britz and Bartsch, 1998), and the apparatus was absent in *Sciaenops ocellatus* and *Mugil cephalus* egg (Li *et al.*, 2000).

There are no particular distinguishing characters between freshwater fish compared to marine eggs and larvae although they have different osmotic problems (Bone *et al.*, 1996), where the freshwater is hypertonic to environment and vice versa in marine fish egg.

Fecundity

The fecundity is essential for studies of population dynamic and life history of fish (Kapoor and Khanna, 2004). In generally fecundity is defined as the number of ripening eggs found in the female just prior to spawning (Bagenal, 1978). The fecundity can be divided into at least six types, namely; potential annual fecundity, annual realized fecundity, total fecundity, relative fecundity, batch fecundity, and annual population fecundity batch or absolute fecundity and relative fecundity.

Potential annual fecundity is the total number of advanced yolked oocytes matured per year (Hunter *et al.*, 1992). Annual realized fecundity is the actual number of eggs finally released. Normally, annual realized fecundity is lower than potential annual fecundity, because some of the eggs were not able to be liberated and remaining in the ovary and being reabsorbed (Murua and Saborido-Rey, 2003). The total or absolute fecundity is the standing stock of advanced yolked oocytes at any time (Hunter *et al.*, 1992), while the batch fecundity is the number of eggs spawned in each batch, and consequently the sum of batch fecundities is the realized annual fecundity. The annual population fecundity is the number of eggs that all the females in a population spawn in a breeding season (Bagenal, 1978). Lastly, the relative fecundity is the number of the standing stock of advanced oocytes at any time compared to body weight (or the number of oocytes per gram body weight).

Based on oocytes recruitment strategy, fecundity can be divided into two type i.e. determinate and indeterminate fecundity. Fish with determinate fecundity, the total fecundity prior to the onset of spawning is considered to be equivalent to the potential fecundity. After correcting for atretic losses, the number of eggs released per female in a year is termed the realized annual fecundity. In batch spawning species the number of yolked oocytes remaining in the ovary decreases with each spawning even (batch) due to the standing stock of yolked oocytes is not replaced during the spawning season (Hunter *et al.*, 1992). While, indeterminate fecundity is refers to species where potential annual fecundity is not fixed before the onset of spawning and unyolked oocytes continue to be matured and spawned during the spawning season (Hunter *et al.*, 1992). The fish with this pattern, the standing stock of previtellogenic oocytes may develop and be recruited into the yolked oocytes stock at any time during the season (Hunter and Goldberg, 1980). Typically, fish with synchronous and group-synchronous pattern indicates that annual fecundity is determinate (Hunter *et al.*, 1992; Greer-Walker *et al.*, 1994).

According to Murua *et al.* (2003) there are at least six methods to estimate the fecundity of fish i.e. gravimetric, volumetric, stereometric, dissector, auto-diametric, and combination among

gravimetric, volumetric, and automated particle counting methods. Each method has advantages and disadvantages and listed in Table 1. However, among available methods, the gravimetric is a popular method due to low cost and time consumed, fast and easy to carry out. The gravimetric has been utilised in some species of fish, for example; *Thynnichthys thynnoides* (Ali and Kadir, 1996), Atlantic sturgeon, *Acipenser oxyrinchus* (Van-Eenennaam *et al.*, 1996), snakehead *Channa striata*, Bloch (Ali, 1999), Baltic cod *Gadus morhua* (Kraus *et al.*, 2000), bagrid catfish *Mystus menurus* (Muchlisin *et al.*, 2006) and Atlantic stargazer *Uranoscopus scaber* (Coker *et al.*, 2008), while the volumetric has been utilised for American eel, *Anguilla rostrata* (Barbin and McCleave, 1997), spotted seatrout *Cynoscion nebulosus* (Brown-Peterson and Warren, 2001), depik *Rasbora tawarensis* (Muchlisin *et al.*, 2011).

Table 1. Advantages and disadvantages of methods used to examine fecundity of fish
(The table was summarized from Murua *et al.*, 2003).

Methods	Advantages	Disadvantages
Gravimetric	Accurate and inexpensive low technology approach, possible to provide additional information on oocytes frequency and oocyte diameter, and very useful technique for batch fecundity estimation	No information on atresia or about presence of spawning markers such as POFs. Not good for species with asynchronous development of oocytes. Requires histological analysis to estimate proportion of atretic to vitellogenic oocytes and to identify presence of POFs, and this method is time consuming.
Volumetric	Inexpensive low technology approach, and possible to provide additional information on oocytes frequency and oocyte diameter	Consider similar with gravimetric
Stereo-metric	Complete analysis includes spawning status, atresia, fecundity, egg size, and number of previtellogenic oocytes. Includes identification of advanced vitellogenic oocytes based on the staining properties and size of oocytes.	Not good for large ovaries >200g, requires expensive high technological instrument expenditure on image analysis. Requires the whole ovary to be returned to the laboratory. Not valid for batch fecundity estimation.
Dissector	Analysis for atresia estimation, section provide histological information including the presence&number of post ovulatory follicle.	Very time consuming and requires serial sectioning of ovary fragment
Auto-diametric	Analysis includes spawning status, fecundity,& egg size. High time efficient method to estimate fecundity. Does not require the whole ovary to be returned to the lab providing saving in space& fixative	Methods published but have to be validated for more species& could be only used for species with determinate fecundity to estimate potential fecundity in prespawning fishes. Not validated for species with asynchronous development oocytes. Requires histological analysis to estimate proportion of atretic to vitellogenic oocytes& identify presence of POFs
Combination between gravimetric/volumetric and automated particle counting	Variation of the gravimetric/volumetric method enables the counting of large amount of oocytes in a short time, which enhances accuracy. Can provide additional information on oocytes size frequency	Difficult to tease oocytes apart, requires Gilson's fixative (toxic) or enzymatic disintegration to tease oocytes apart. No information on atresia or about presence of spawning markers such as POFs. Requires histological analysis to estimate proportion of atretic to vitellogenic oocytes and to identify presence of POFs. Expensive electronic equipment necessary and calibration.

There is strong relationship between absolute fecundity and length, weight or age of fish. However, the absolute fecundity is more likely to be more closely correlated with weight than length (Ali and Kadir, 1996), but where the correlations have been analysed adequately very little advantage has been found in considering weight rather than length and numerous problems arise. In most of fish the somatic weight changes significantly toward spawning, when the weight used in the correlations is total weight (somatic+gonad) a spurious correlation may be obtained since the greater number of eggs in the more fecund fish will weigh more than those in the less fecund (Bagenal, 1978). Therefore, a gonad-free body weight is more acceptable to be used to analyse the relationship between absolute fecundity and somatic weight.

Generally, absolute fecundity increases with increasing broodfish size (Buckley *et al.*, 1991; Bromage *et al.*, 1990; Bone *et al.*, 1996), but the egg size may vary from one spawning to another, and the number of eggs contained in a specific volume may also be different (Carrillo *et al.*, 2000; Jonsson and Jonsson, 1997) and the fecundity does not increase after a certain stage in maturation, so the fecundity for a given length may appear to decrease, although the fecundity of any individual fish has remained the same (Bagenal, 1978).

The positive relationship between fecundity and body size has been reported in some species, for example in salmonids and brown trout (Jonsson and Jonsson, 1999; Jonsson *et al.*, 1996). A similar finding was also reported in cardinalfish, *Apogon lineatus* (Kume *et al.*, 2000). Jonsson and Jonsson (1997) argued that increase of body size will increase the body cavity to accommodate more eggs and more energy available to produce many eggs. Beside that water temperature are factor indentified to influence the relative fecundity of Baltic cod *Gadus morhua* (Kraus *et al.*, 2000). However, there was an inverse relationship between fecundity and egg size (Bone *et al.*, 1996), thus female producing larger eggs were limited to produce fewer eggs (Matthews, 1998). In species whose eggs develop in open waters or are freely scattered on the bottom, population strength is usually maintained by high fecundity relative to the small egg size. Development of adaptations for care of progeny is accompanied by decreased fecundity and usually increased egg size with greatly increase energy resources for embryo and enables the embryo to develop without obtaining exogenous food, and to reach active life at a higher level of differentiation and ensuring higher survival (Ginzburg, 1972).

On the other hand, the relative fecundity is based on weight of fish. In most fish the number of eggs does not change significantly as the season progresses and the relative fecundity is constant through the season (Bagenal, 1978). For comparison fecundity among fishes, the relative fecundity is commonly used.

Gonadal Development and Spawning Frequency

Gonadal development

The gonadal development of fishes is affected by various factors i.e. genetics, nutrition, broodfish and environmental conditions. The comprehensive of understanding on factors affect the reproductive biology of fishes have been reviewed by Muchlisin (2005).

In general, the gonadal development or maturity stage of fishes could be evaluated macroscopically and microscopically. Microscopic method was assessed based on histological appearance and egg size distribution using microscope magnification, while macroscopic analysis involved eye-naked observation of gonads such as gonad weight (gonadosomatic index, GSI), gonad colour and other morphological performances of gonad. Generally the evaluation involved both method to obtain the best describes of the gonads.

Gonadosomatic index (GSI) is the ratio of gonad weight to body weight. Total body weight was used by some researchers to assess GSI of fishes for example cardinalfish, *Apogon lineatus* (Kume *et al.*, 2000), while the Brown-Peterson *et al.*, (2001) and Brown-Peterson and Warren (2001) have used the netto weight with excluded the gonad weight in their evaluation. The both methods mentioned were formulated as follow:

- $GSI = GW / (TW - GW) \times 100$
- $GSI = GW / TW \times 100$

where GSI is gonadosomatic index, GW is gonad weight, TW is total weight. The first method was used in our study previous study with the reason that the first method more accurate because the used data is gonad-free bodies weight and probably no bias occurring, this method was also used by Muchlisin *et al.* (2011) in assessing the fecundity of *R. tawarensis*.

Based on both examinations: macroscopic and microscopic, the gonadal development can be divided onto some stages. For instance, Ali and Kadir (1996) has classified the maturity stage of *T. thynnoides* into four stages i.e. immature, maturing, ripening and spent. While Nichol (2001) has classified the maturity stage of female yellowfin sole into five stages i.e. immature, maturing, hydrated, and spawning and spent. Marcano *et al.* (2007) has used a single method of macroscopic to determining the maturity stages of the gonad *Oxydoras sifontesi* and it was classified into six stages i.e. virgin, immature, maturing, mature, spermiated/ovulated and spent/spawned. Alonso-Fernandez *et al.* (2008) have classified gonadal development of female pouting *Trichopterus luscus* into two stages i.e. immature and mature stages, and then divided mature stage into six sub stages ripening, recently- spawned, spawning-hydrated, partly spent, inactive mature, and recovering. In addition, Grandcourt *et al.* (2009) have examined the reproductive biology of grouper *Epinephelus coioides* and classified female gonad into six stages i.e. immature, undetermined inactive, mature resting, mature ripe, mature running ripe, and spent. We used five levels of gonada developmental stage for depik, *R. tawarensis* i.e. immature, develop, mature, ripe and spent (Muchlisin *et al.*, 2010, Table 2).

Table 2. The gonad developmental stages of *R. tawarensis* and its description based on macroscopic evaluation (the table was cited from Muchlisin *et al.*, 2010)

Stage	Classification	Gonad appearance		GSI range		Oocyte size (μm)
		Testes	Ovary	Female	Male	
I	Immature	Small, flat, translucent to whitish, poorly developed, with reduced fringes.	Small, transparent to translucent and not very voluminous. Oocyte not visible with naked eye.	< 10.9	< 2.0	209.61 - 592.78 (447.30)
II	Develop	Whitish with voluminous fringes.	Large orange-pale, oocytes may be visible through the ovary tunic.	11.0 - 18.9	2.5 - 5.5	528.37 - 867.10 (711.24)
III	Mature	Very large, firm, white in colour.	Very large occupying part of the abdominal cavity. Yellow oocyte turgescency	19.0 - 23.9	5.6 - 8.0	604.15 - 894.33 (780.59)
IV	Ripe	Fully developed, turgid fringes, milky-whitish in colour. Milt run out of the fish.	Occupying the entire abdominal cavity. Ovulated oocytes can be fully expelled from the oviduct with gentle pressure.	>24.0	> 8.0	725.27 - 991.81 (844.09)
V	Spent	Bloody and flaccid fringes.	Flaccid, red-brown or bloody in colour. Few remaining large oocytes observed, and smaller size oocytes may be seen.	2.5 - 10.9	1.5 - 3.0	227.64 - 770.82 (447.38)

Spawning frequency

Spawning frequency is the number of days between spawning. According to Brown-Peterson *et al.* (2001) that spawning frequency was determined based on histological observation and two methods were utilized i.e. (a) the percentage of female in the late developing ovarian class with 0-h to 24-h postovulatory follicle (POF) in the ovary and (b) the percentage of female in the late developing class undergoing final oocyte maturation (FOM). Furthermore Brown-Peterson *et al.* (2001) stated that spawning frequency was determined by dividing 100 (representing the total population of fish) by the percentage of fish with FOMs or POFs in the ovaries.

The timing of spawning of Southeast Asian cyprinids is usually related to the annual rainfall cycle of the seasonal tropics (Rainboth, 1991). Many studies have reported high correlation of rainy season with spawning peaks of fishes. For instance, Solomon and Ramnarine (2007) reported that the spawning season of white mullet *Mugil curema* from the Southern Caribbean coincided with peak rainfall in June, as indicated by a maximum GSI value in this period. Adite *et al.* (2006) also reported the spawning season of the African bonytongue fish in the So River in the floodplain of West Africa occurred during the wet season (May to August) as floodwaters gradually rise. In addition, the reproduction in *Tor putitora* was observed mainly in autumn months of March to April and also in the monsoon months, from July to August. During the months, *T. putitora* was migrated from the main river to the tributaries where it bred in the flooded waters (Singh, 2007). Currently Muchlisin *et al.* (2010) reported the gonadosomatic indices (GSI) of female and male depik, *R. tawarensis* were highest in the months of March, September and December with the peak in September, indicating the onset of the reproductive seasons during the rainy season.

In conclusion, in most teleosts, ovarian development and the ultimate production of mature eggs is a highly complex process, time and modulated by various environmental and endocrine pathways such young are produced only at times when fry survival is optimal, usually when food availability is at its highest (Coward and Bromage, 2000).

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